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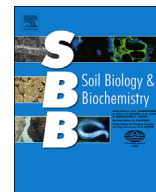
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# Understanding the legacy effect of previous forage crop and tillage management on soil biology, after conversion to an arable crop rotation



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## ABSTRACT

The soil ecosystem provides a habitat for numerous and diverse fauna which hold a pivotal role driving decomposition and nutrient cycling. However, changing land use or management can alter population dynamics, changing soil biology within the system. The implementation of different field management can improve soil fertility, whilst natural variations in plant species growth and root system may create changes to soil structure and properties. All plant species create a legacy effect within the soil to some extent; changing the environment either physically or through remaining plant residues. An experiment investigated the hypothesis that previous forage cropping and tillage management would alter the diversity and abundance of soil fauna, after changing from a stable soil environment for three years to an annual arable crop rotation to complete a five-year rotation cycle. Four replicate plots (crop 1) of either perennial ryegrass (*Lolium perenne*), red clover (*Trifolium pratense*), white clover (*Trifolium repens*) or chicory (*Cichorium intybus*) were grown in a randomised block design (2009–2013) as the first crop, before conversion to an arable crop rotation. Spring wheat (*Triticum aestivum*) was established in 2013, either by conventional ploughing (CP) or direct drilling (DD); and winter barley (*Hordeum vulgare*) established using the same methodology the following autumn 2013 and harvested in 2014. Soil fauna abundance was sampled each year after the cereal crop was harvested, and included microfauna (nematodes), mesofauna (mites) and macrofauna (earthworms). Nematodes were found in greatest abundance in the previously ryegrass treatments, with greater numbers of bacterial feeders and herbivores (in 2013). Mesostigmata and oribatid mites had larger abundances in the ryegrass treatments, although Prostigmata were found in numbers five times higher after red clover in DD plots (in 2013); earthworms were found in significantly greater numbers in the previously white clover plots, across both cereal crops. These legacy effects began to diminish by the end of the second cereal crop in the rotation (in 2014). Tillage management also affected abundance, although these were fauna dependent, with earthworm numbers being detrimentally affected by ploughing whilst nematode abundances increased with ploughing. The combination of legacy and tillage elucidated interactions with the different groups of fauna, for example, epigeic earthworms, wireworms, and prostigmatid mites showed changes in abundance dependent on the combined effect of forage and tillage. Overall, legacy effects were found across three organism scales, highlighting the impact agricultural cultivations have across the whole soil food web.

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## 1. Introduction

Demand for food production is increasing globally and the need for it to be a sustainable intensification of agriculture (Garnett et al., 2013) is also increasing. Understanding how to increase yields with minimum degradation to soil structure and function is key to agriculture in the long term. The global need to provide increased crop yields with minimal environmental impact (Ball et al., 2005)

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has led to our investigation of arable crop rotation after forage leys, as a potential soil improving cropping system to promote soil health and consequently soil biodiversity. Soil invertebrates are one of the best indicators of soil quality as changes in soil properties affect both composition and abundance (Lavelle et al., 2006). Agricultural grasslands (permanent pasture) support a relatively stable and numerous soil biota that contribute to soil functioning and fertility (Murray et al., 2012). However, arable crops are not considered to be a stable environment, as plant species change from a perennial to an annual, eliminating soil fauna that are susceptible to damage, desiccation and destruction of microhabitats (Behan Pelletier, 2003).

Soil fauna are classified across a range of scales within the soil food web that span three orders of magnitude (Swift et al., 1979). Nematodes (microfauna) are extremely diverse in both species and function, (feeding on bacteria, fungi, plants, as well as being omnivorous and predatory) (Yeates et al., 1993); they also play a large role in nutrient cycling and microbial turnover (Neher, 2001; Bonkowski, 2004). Mesofauna are key mediators of soil function, including the comminution and incorporation of litter, as well as regulating microbial communities (Lavelle et al., 2006). Earthworms have the largest effect as they are ecosystem engineers (Jones et al., 1994) changing both the physical structure of the soil habitat as well as altering its chemical composition (Blouin et al., 2013). However, when agricultural grassland becomes part of an arable crop rotation, the intensity of land use alters the stability of the environment and loss of biodiversity, and reductions in abundance have been shown to occur (Firbank et al., 2008). Soil fauna have been found to be negatively affected by the intensity of agriculture (fertiliser inputs/crop rotation) (Ponge et al., 2013); with, high inputs of inorganic fertiliser and increased tillage promoting bacterial feeding organisms, whilst low inputs and minimum tillage, promotes fungal feeding organisms (De Vries et al., 2012). However, considering the changes in agriculture as part of an arable crop rotation, negative effects may be buffered by the legacy of the previous cropping system (Detheridge et al., 2016).

Forage crops may buffer the impacts of grassland conversion into arable crops by altering the overall resilience of the soil in relation to change. For example, to obtain maximum yields from ryegrass swards, inorganic fertilisers are applied regularly whilst legumes fix atmospheric N (Carlsson and Huss-Danell, 2012) reducing the intensity of management. The addition of inorganic fertiliser will leave a different legacy to the leguminous forages which are known to leave residual N for future crop uptake (Kirkegaard and Ryan, 2014). Different grassland species have variable concentrations of essential nutrients and different rooting patterns, all potentially affecting the soil environment. Chicory, for example, has a deep tap rooting system that has been found to mine micronutrients from the soil, changing the location of nutrients within the soil profile (Belesky et al., 2001). Variability among rooting systems and plant cover between species leads to differences in productivity, the stability of soil, changing microbial processes (White et al., 2013) and affects the soil food web itself.

Our previous work has shown that earthworm abundance was higher in white clover than other forages as well as increasing herbivore abundance of invertebrates within ryegrass compared to chicory and clovers (Crotty et al., 2015). However, we do not understand the legacy effects of the previous forage crops on the succeeding crop within a rotation in relation to soil biology. Legacy effects, or ecological inheritances (Han et al., 2014), are the impact of historical management or perturbation that continues to affect ecosystem structure and function. Crop rotation alters the soil ecosystem, either directly through the cultivation of the soil or, indirectly, via the replacement of perennial plants with annual crops (DuPont et al., 2010). Agricultural grassland is commonly

changed to become part of an arable crop rotation, with wheat often followed by barley in rotation (BIO Intelligence Service, 2010). Different tillage regimes can also be used to effectively prepare the seedbed and sow the following crop. Tillage (ploughing) is known to be detrimental to soil fauna, particularly earthworms (Bertrand et al., 2015); Collembola (Bedano et al., 2006); Acari (Behan Pelletier, 2003) and to a lesser extent Nematodes (Fiscus and Neher, 2002). It is also unknown whether there will be an interaction between the legacy effect of a previous forage crop and the method of establishment used e.g. tillage or direct drill.

This study examined the legacy effects from four preceding forage crops, (perennial ryegrass (*Lolium perenne*) low N fertiliser or 200 kg N fertiliser per annum, red clover (*Trifolium pratense*), white clover (*Trifolium repens*) or chicory (*Cichorium intybus*)), on soil fauna after spring wheat (*Triticum aestivum*) established either by conventional mould-board ploughing (CP) or by direct drilling (DD) inverted T, coulter drill; and, followed by winter barley (*Hordeum vulgare*) established by the same methods. Will legacy effects be found to affect soil fauna abundance after the first cereal crop, will they remain and differ between the different tillage managements after a second cereal rotation? Will the tillage management (ploughing or direct drill) affect all soil fauna in similar ways independent of forage legacy effects?

## 2. Materials and methods

### 2.1. Experimental site, plot establishment and maintenance

#### 2.1.1. Crop 1: original pure sward forages

A full description of the experimental methods regarding the previous forage crops was presented in Crotty et al. (2015). In brief, the experimental area was set up at Institute of Biological, Environmental and Rural Sciences (IBERS), Aberystwyth University, Wales (52°25' 59"N, 4°1' 26"W) in 2009; plots were uniformly ploughed to the same depth (175 mm) and standardised in accordance with UK farming guidelines (RB209; DEFRA, 2010). Replicated plots (7.5 m × 12 m) of five forage treatments were set up on an area of stony, well-drained loam of the Rheidol soil series in a randomised block design (n = 4). Perennial ryegrass (*Lolium perenne*) (cv. Premium) with minimal input of inorganic N ha<sup>-1</sup> (80 kg N ha<sup>-1</sup> only, applied in three years) (PRG Low N), perennial ryegrass plus 200 kg inorganic N ha<sup>-1</sup> annum<sup>-1</sup> (PRG 200N), chicory (*Cichorium intybus*) (cv. Puna II) (CH), white clover (*Trifolium repens*) (cv. AberDai) (WC) and red clover (*Trifolium pratense*) (cv. Merviot) (RC). All crops were mechanically harvested regularly to simulate a silage cutting system, experimental maintenance and forage sampling was as described in Marley et al. (2013).

#### 2.1.2. Crop 2: spring wheat (*Triticum aestivum*)

In February 2013, 360 g l<sup>-1</sup> glyphosate at 4 l ha<sup>-1</sup> (Gallup 360 herbicide, Barclay Ltd, Dublin, Ireland) was applied to all plots. Each plot was split (3.75 m × 12 m) into two sub-plots and allocated at random to two tillage treatments (either conventional ploughed (CP) or no-till direct drilled (DD)). Sub-plots CP were mould board ploughed to a depth of 175 mm and power-harrowed, whilst those DD were undisturbed prior to sowing. Spring wheat (cv Tybalt) was sown using a Duncan Ecoseeder (Duncan Ag, Timaru, NZ) at a rate of 253 kg ha<sup>-1</sup>, on all plots on the 5th of April, and flat rolled. This Ecoseeder has an inverted T type coulter drill forming a slot to sow the seed creating minimal soil disturbance. Spring wheat was harvested on 29th August 2013, using a Sampo harvester and the grain and straw removed.

#### 2.1.3. Crop 3: winter barley (*Hordeum vulgare*)

Following the harvest of spring wheat, all plots were treated

with herbicide (360 g l<sup>-1</sup> glyphosate at 4 l ha<sup>-1</sup>) on the 10th October 2013. Sub-plot tillage treatments remained the same for the barley as for wheat. Thus CP sub-plots were mould board ploughed and power-harrowed 14th October 2013, prior to sowing. Winter barley (cv. Pearl) was sown on all subplots on the 15th October 2013, at the rate of 196 kg ha<sup>-1</sup>, as for the previous wheat establishment, except all plots were Cambridge rolled and no fertiliser was applied at this stage of establishment. Standard application of lime, fertiliser, herbicides and pesticides were applied (for both wheat and barley) (Rhymes et al., 2016). Winter barley was harvested on the 15th July 2014, using a Sampo harvester, with grain and straw removed. The total cereal offtakes (for both wheat and barley) and the relationship to soil chemistry are presented in Rhymes et al., 2016.

## 2.2. Soil fauna sampling

The pure sward forages were first sampled in autumn 2012 (results found in Crotty et al., 2015). Here, we present the first sampling taken after one year of wheat rotation (autumn 2013) after the harvest. The second sampling date was after the barley rotation, two years of arable crops (summer 2014), after the winter barley was harvested. The abundance of key functional groups in the soil food web, including earthworms, nematodes and microarthropods were quantified. Soil was taken from randomised areas within each plot for each of the faunal groups. The areas where earthworm samples were taken were randomised and recorded so that the same area was not resampled the following year due to the size of the sample compared to the other faunal measures and the need for the direct drilled plots to remain undisturbed.

### 2.2.1. Earthworm population assessment

Earthworm biomass (g m<sup>-2</sup>), abundance (m<sup>-2</sup>) and diversity were quantified from within a square of soil (30 cm by 30 cm) excavated to a depth of 30 cm (Schon et al., 2014), from each plot and taken from the field to be hand-sorted and identified in the laboratory. To ensure deep burrowing earthworms were also counted, after excavation, 0.5% formaldehyde (Merck, Poole, UK) solution was added to the pit to expel the deep burrowers, these were removed, washed and added to respective sample. The Simpson index of diversity (1-D) was also used to measure differences in earthworm species composition. Other macrofauna abundance and diversity were also quantified from the soil block taken for earthworm sampling. These were identified and counted and included beetle larvae, fly larvae, centipedes, millipedes and adult beetles.

### 2.2.2. Nematode population assessment

Twenty soil samples per plot were collected to a depth of 15 cm (Ferris and Matute, 2003) from across each sub-plot using a 4 cm diameter auger. A 200 g soil sub-sample was taken to assess dry weight by oven drying at 105 °C for 24 h. A 200 g sub-sample of fresh soil was used for nematode wet tray extractions (Whitehead and Hemming, 1965) adapted by Crotty et al. (2011). The total number of nematodes per metre squared was calculated using soil bulk density data for each treatment obtained at each sampling point (following the methods of Sylvain et al. (2014)) and multiplied by the depth the sample was taken from (15 cm). Samples were reduced through siphoning, to 5 ml, transferred to sample tubes with an equal volume of 8% formaldehyde to preserve samples until identification to functional groups was performed. Nematode feeding functional groups were identified as described in Crotty et al. (2015); adapting the method of Freckman and Ettema (1993), using an inverted compound microscope.

### 2.2.3. Microarthropod sampling

Microarthropods were sampled from three intact soil cores (5 cm diameter, 10 cm depth (Murray et al., 2009)) collected from each sub-plot (N = 40) and placed together upside down on Tullgren funnels for extraction over seven days. Invertebrates were collected into 20 ml vials containing 70% ethanol, where they were stored until being counted and identified, separating to the lowest taxonomic resolution of the main superfamilies/lineages for Collembola and mites (Hopkin, 2007; Krantz and Walter, 2009) as well as identifying the other invertebrates to order (Tilling, 1987). The Simpson index of diversity (1-D), a measure of community composition was also used to compare the microarthropods extracted.

### 2.2.4. Statistical analysis

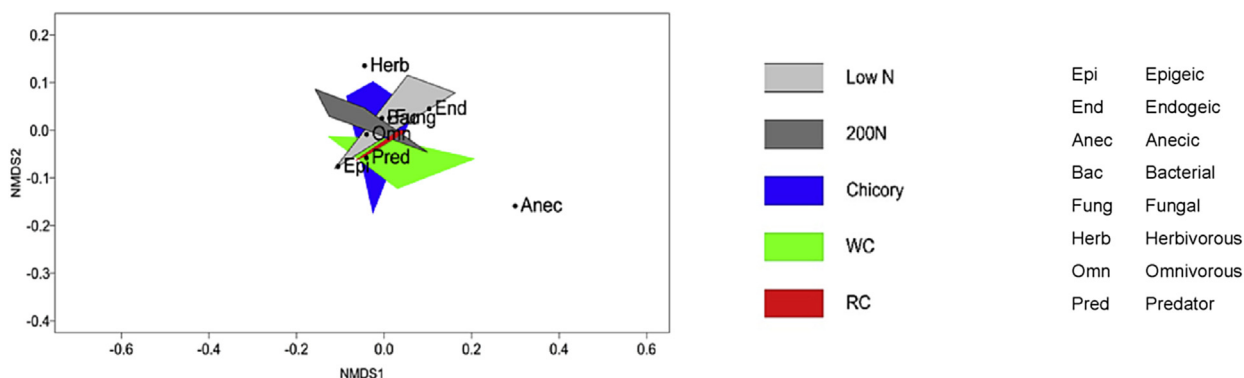
All data were analysed in GenStat® (Payne et al., 2014), with a split plot design, analysed by repeated measures and effects of previous forage treatment estimated in the whole plot stratum and effects of tillage and forage-tillage interactions estimated at the sub-plot level. Data were normalised prior to univariate and multivariate analysis of variance when necessary. When there were significant differences between the effects of previous forage treatment, multiple comparisons were made using the Student Newman Keuls test (SNK) following the methods described by Thomas (1973). Where an interaction between tillage management and forage treatment was found comparisons were restricted to between forages within tillage management and between tillage management within forage with comparison-wise type I error rate adjusted using the Bonferroni approximation (Abdi, 2007). Spearman's rank correlation coefficient was used to assess the relationship between different orders of macrofauna. The Simpson index of diversity (1-D), a measure of community composition was assessed for all earthworms species and for all microarthropods extracted via the Tullgren funnels (including Collembola and Acari main superfamilies/lineages, Coleoptera larvae and adults, Diptera larvae and adults, Enchytraeidae, Lumbricidae, Hemiptera, Araneae, Chilopoda, Diplopoda, Pauropoda, Protura, Symphyla and Thysanoptera) separately. This was calculated from the equation:  $1 - \sum_{i=1}^s n_i(n_i - 1) / (N(N - 1))$ , where  $n_i$  is the number of organisms of species  $i$  and  $N$  the total number of organisms of all  $s$  species within each habitat.

To understand how the community assemblages changed over the crop rotation, according to tillage management, non-metric multidimensional scaling (NMDS) analysis was carried out in R (R Core Team (2015); Version 3.1.3) using package vegan (Oksanen et al. (2015)) and using data for the main functional groups within the earthworm and nematode orders; three earthworm groups (epigeic, endogeic, and anecic) and five nematode groups (bacterial, fungal, herbivore, omnivore and predatory), as well as population numbers for the earthworm and nematode functional groups obtained by Crotty et al. (2015) in autumn 2012. The analyses were used first to examine how the populations changed post wheat and then post barley, with the distances between the convex hulls representing the degree of similarity between the communities studied.

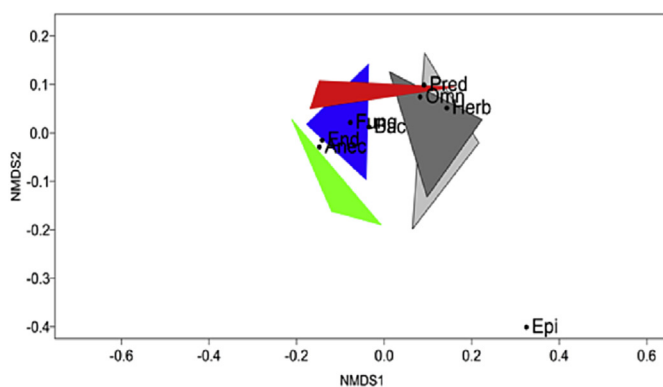
## 3. Results

The NMDS plots highlight how the community assemblage changed during the two years of crop rotation (Fig. 1). Within the graphical configuration of the NMDS plots, functional groups that are close to each other co-exist at similar abundances within the different forage treatments. From the original functional group composition (Fig. 1a)) anecic earthworms are showing the greatest variation, this continues within the wheat CP (Fig. 1c)) and both

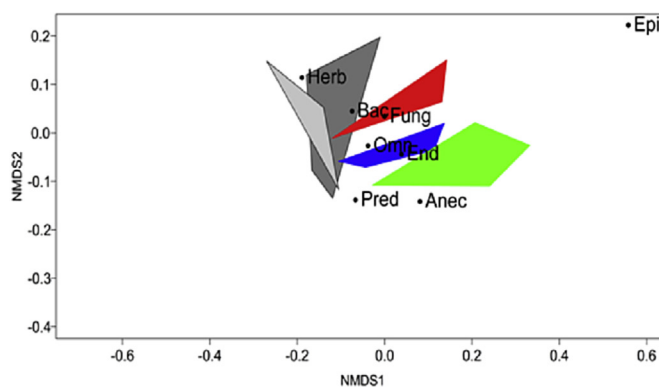
## a) Forage



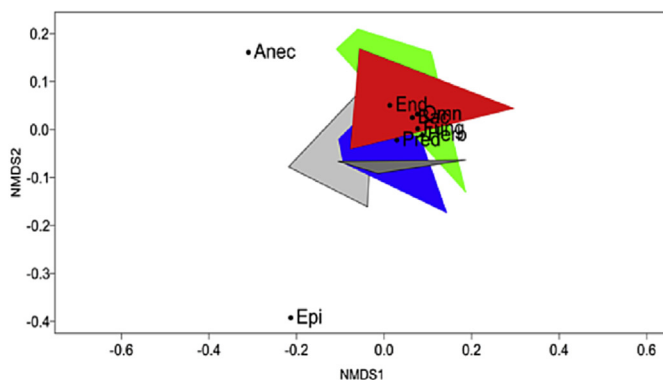
## b) Wheat DD



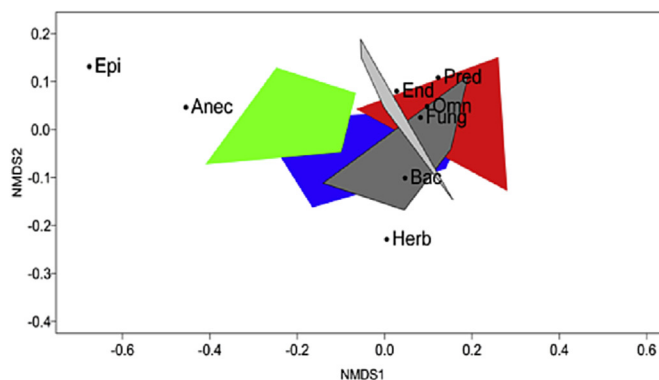
## c) Wheat CP



## d) Barley DD



## e) Barley CP



**Fig. 1.** Non-metric multidimensional scaling (NMDS) using data for the three earthworm (Epi, End and Anec) and five nematode (Bac, Fung, Herb, Omn and Pred) functional groups a) post forage, b) post wheat direct drilled, c) post wheat ploughed, d) post barley direct drilled and e) post barley ploughed.

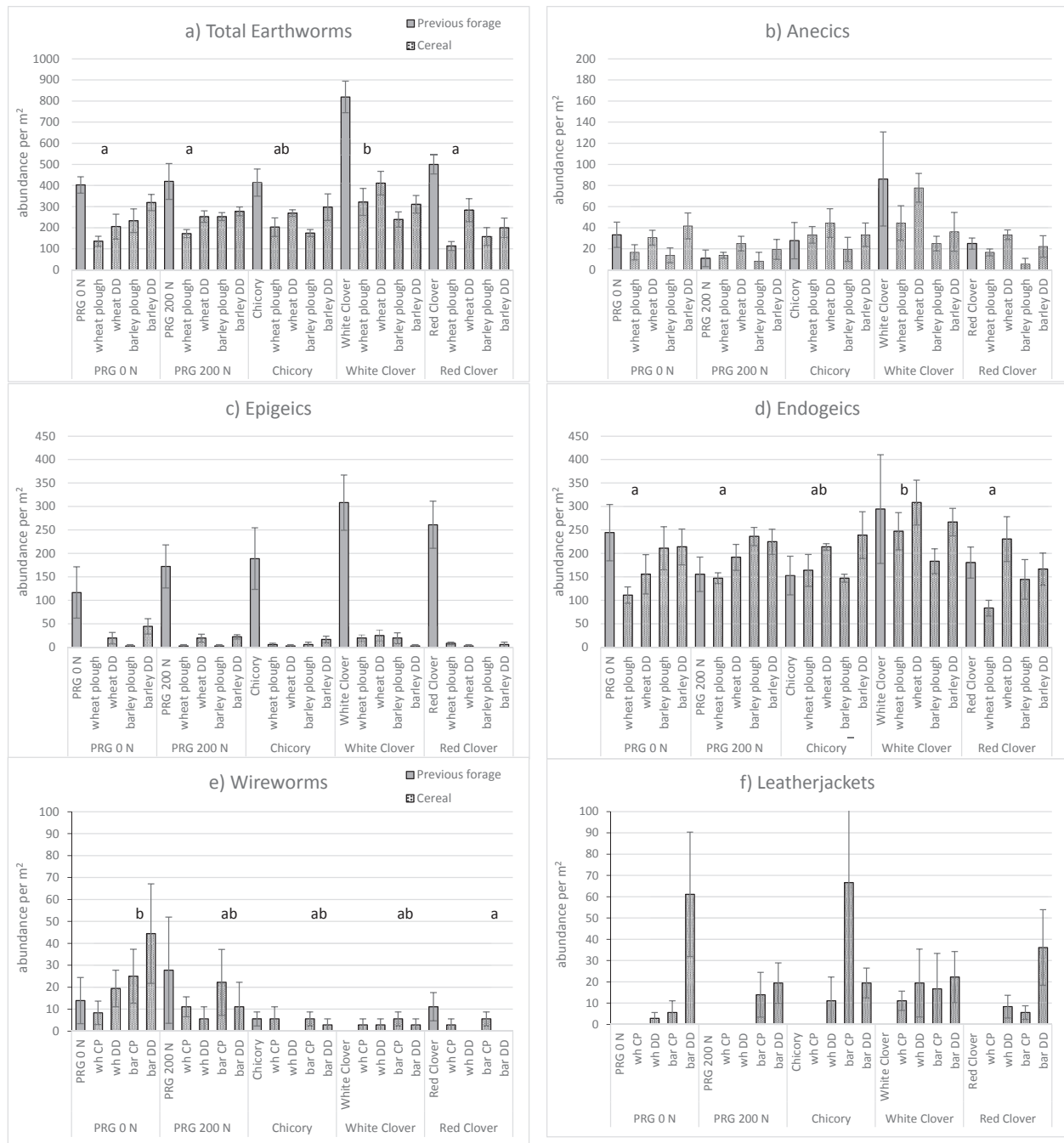
barley plots (Fig. 1d) and e)). The epigeic earthworm group also begins to diverge greatly over the two cereal groups particularly after CP (Fig. 1c) and e)). Although there is a large overlap of the forage convex hulls within Fig. 1a), the differences between the previous forage treatments become greater after the wheat rotation (particularly after CP) as there is less overlap (Fig. 1b) and c)). After barley DD this effect diminishes as can be seen due to the greater overlap (Fig. 1d)) whereas barley CP continues to show large differences between the previous forage treatments (Fig. 1e)).

### 3.1. Earthworm population assessment

There was a change in earthworm abundance over time, with a

significant reduction in earthworm numbers across all forages for both cereal crops and establishment methods compared to previous forage (Fig. 2(a)), this was greatest for the CP treatments but was still present in DD. The change in earthworm abundance for two of the earthworm functional groups was less (Fig. 2(b) and (d)), with either an initial reduction after wheat before a recovery after barley or relatively similar levels over time, with tillage being a more important factor. There was a large reduction in population numbers for the epigeic earthworms (Fig. 2(c)) after the cereal crops compared to the previous forage. Total earthworm numbers over all, as well as the number of epigeic and endogeic earthworms, showed an interaction between forage treatment and cereal crop. Post wheat, total earthworm numbers, epigeic and endogeic were





**Fig. 2.** Change in abundance over time per m<sup>2</sup> (untransformed mean  $\pm$  standard error) for total earthworms (a), anecic earthworms (b), epigeic earthworms (c), endogeic earthworms (d), wireworms (e) and leatherjackets (f), post forage, post wheat ploughed (wh CP) and direct drilled (wh DD) and post barley ploughed (bar CP) and direct drilled (bar DD). Multiple comparisons based on bonferroni adjusted multiple comparisons ( $P < 0.05$ ), a, b indicate forage means differ within crop (CP and DD combined).

more abundant in WC plots than in Low N, 200N and RC plots ( $P = 0.008$ ,  $P = 0.044$  and  $P = 0.047$  respectively; [Table 1](#), [Supplementary Table S.1](#) and [Fig. 2\(a\)](#), (c) and (d)) but post barley differences between forages were not detected ( $P > 0.05$ ). Anecic worms were marginally but not significantly ( $P = 0.065$ ) more abundant in WC plots post wheat but were more abundant post wheat than post barley ( $P = 0.002$ ; [Fig. 2\(b\)](#)). This signifies the legacy effect differed between cropping years, with the wheat crop having greater differences in earthworm abundances across the different forages than after barley. Earthworms were overall more abundant in direct drilled plots than in ploughed plots ( $P = 0.001$ ) and within each functional group, epigeic ( $P = 0.020$ ), endogeic

( $P = 0.006$ ) and anecic ( $P = 0.017$ ) ([Table 1](#)). However, earthworm biomass results showed that this effect was not as prominent after barley, as it was in wheat – with significant differences between total biomass within the different crops but the same tillage method ( $P = 0.011$ ; [Supplementary Table S.1](#)). Simpson's index of diversity was not affected by previous forage but showed an interaction between tillage method and cereal crop ( $P = 0.025$ ) with values for CP and DD plots of 0.646 and 0.625 respectively post wheat ( $P > 0.05$ ) and significantly greater diversity ( $P < 0.05$ ) in DD plots (0.693) post barley (compared to CP 0.609) ([Supplementary Table S.1](#)).

The two most commonly found other macrofauna were

**Table 1**  
Highlights of normalised mean abundance (earthworms, nematodes ( $n \times 10^6$ ) and mesofauna  $n \text{ m}^{-2}$ ) after wheat then barley cultivation (Crop (C)) following different forage treatments (F) and established by either direct drilling (DD) or after ploughing (CP) (Tillage (T)). Multiple comparisons based on bonferroni adjusted multiple comparisons ( $P < 0.05$ ). <sup>a, b</sup> indicate forage means differ within crop, <sup>A, B</sup> indicate tillage means differ within crop and \* indicate tillage means within crop differ.

|                               | Crop (C) | Forage (F)          |                     |                                |                    |                      | Mean   | Effect | Prob         | Effect | Prob             | Effect | Prob             | Tillage effect |
|-------------------------------|----------|---------------------|---------------------|--------------------------------|--------------------|----------------------|--------|--------|--------------|--------|------------------|--------|------------------|----------------|
|                               |          | Low N               | 200N                | Chicory                        | WC                 | RC                   |        |        |              |        |                  |        |                  |                |
| Total                         | Wheat    | 170.8 <sup>a</sup>  | 212.5 <sup>a</sup>  | 236.1 <sup>ab</sup>            | 366.7 <sup>b</sup> | 198.6 <sup>a</sup>   | 236.9  | F      | <b>0.022</b> | C      | 0.567            | T      | <b>0.001</b>     | DD > CP        |
| Earthworms                    | Barley   | 276.4               | 265.3               | 236.1                          | 275.0              | 179.2                | 246.4  | FxT    | 0.939        | FxC    | <b>0.008</b>     |        |                  |                |
| Epigeic <sup>Sx</sup>         | Wheat    | 5.4                 | 5.4                 | 2.1                            | 17.5               | 4.6                  | 6.4    | F      | 0.215        | C      | 0.609            | T      | <b>0.020</b>     | DD > CP        |
| Earthworms                    | Barley   | 12.9                | 9.6                 | 7.9                            | 6.5                | 1.6                  | 7.3    | FxT    | 0.056        | FxC    | <b>0.044</b>     |        |                  |                |
| Endogeic                      | Wheat    | 135 <sup>a</sup>    | 178 <sup>a</sup>    | 192 <sup>ab</sup>              | 279 <sup>b</sup>   | 157 <sup>a</sup>     | 188    | F      | <b>0.018</b> | C      | 0.280            | T      | <b>0.006</b>     | DD > CP        |
| Earthworms                    | Barley   | 212                 | 231                 | 193                            | 225                | 156                  | 203    | FxT    | 0.618        | FxC    | <b>0.047</b>     |        |                  |                |
| Anecic <sup>S</sup>           | Wheat    | 20.1                | 17.9                | 34.9                           | 51.5               | 23.4                 | 27.9   | F      | 0.065        | C      | <b>0.002</b>     | T      | <b>0.017</b>     | DD > CP        |
| Earthworms                    | Barley   | 21.4                | 9.1                 | 18.4                           | 23.4               | 9.1                  | 15.6   | FxT    | 0.945        | FxC    | 0.404            |        |                  |                |
| Wireworm                      | Wheat    | 10.9 <sup>*</sup>   | 6.3                 | 1.6                            | 2.1                | 1.0                  | 4.0    | F      | <b>0.023</b> | C      | 0.058            | T      | 0.154            | -              |
| ( <i>Elateridae</i> )         | Barley   | 25.1 <sup>b</sup>   | 9.5 <sup>ab</sup>   | 3.3 <sup>ab</sup>              | 3.3 <sup>ab</sup>  | 2.1 <sup>a</sup>     | 7.2    | FxT    | <b>0.012</b> | FxC    | 0.645            |        |                  |                |
| Leatherjackets                | Wheat    | 1.0                 | 0                   | 2.5                            | 10.0               | 2.8                  | 2.9    | F      | 0.781        | C      | <b>0.001</b>     | T      | <b>0.007</b>     | DD > CP        |
| ( <i>Tipulidae</i> )          | Barley   | 17.7                | 11.6                | 14.9                           | 11.1               | 13.8                 | 13.7   | FxT    | 0.444        | FxC    | 0.555            |        |                  |                |
| Total                         | Wheat    | 13.96 <sup>ab</sup> | 14.39 <sup>b</sup>  | 11.53 <sup>ab</sup>            | 11.03 <sup>a</sup> | 12.62 <sup>ab</sup>  | 12.67  | F      | 0.052        | C      | 0.118            | T      | <b>0.002</b>     | CP > DD        |
| Nematodes <sup>‡</sup>        | Barley   | 12.99               | 11.24               | 13.21                          | 11.82              | 10.16                | 11.85  | FxT    | 0.320        | FxC    | <b>0.023</b>     | TxC    | <b>0.028</b>     |                |
| Bacterial feeding             | Wheat    | 5.01 <sup>ab</sup>  | 5.86 <sup>b</sup>   | 4.60 <sup>a</sup>              | 3.72 <sup>ab</sup> | 4.80 <sup>ab</sup>   | 4.75   | F      | 0.174        | C      | <b>0.007</b>     | T      | 0.771            | –              |
| Nematodes <sup>‡</sup>        | Barley   | 3.67                | 4.19                | 4.37                           | 4.39               | 3.45                 | 4.00   | FxT    | 0.660        | FxC    | <b>0.042</b>     | TxC    | <b>0.021</b>     |                |
| Fungal feeding                | Wheat    | 4.98                | 4.36                | 4.88                           | 5.72               | 6.03                 | 5.16   | F      | 0.072        | C      | 0.089            | T      | <b>&lt;0.001</b> | CP > DD        |
| Nematodes <sup>‡</sup>        | Barley   | 5.64                | 4.05                | 5.15                           | 4.30               | 4.15                 | 4.62   | FxT    | 0.571        | FxC    | 0.084            |        |                  |                |
| Herbivore feeding             | Wheat    | 2.32 <sup>b</sup>   | 2.43 <sup>b</sup>   | <sup>A</sup> 0.90 <sup>a</sup> | 0.41 <sup>a</sup>  | 0.67 <sup>a</sup>    | 1.15   | F      | <b>0.005</b> | C      | 0.192            | T      | 0.669            | –              |
| Nematodes <sup>‡</sup>        | Barley   | 1.44                | 1.33                | <sup>B</sup> 1.59              | 1.44               | 0.97                 | 1.34   | FxT    | 0.316        | FxC    | <b>&lt;0.001</b> |        |                  |                |
| Omnivorous                    | Wheat    | 0.55                | 0.67                | 0.33                           | 0.42               | 0.38                 | 0.45   | F      | 0.096        | C      | 0.331            | T      | 0.702            | –              |
| Nematodes                     | Barley   | 0.70                | 0.48                | 0.50                           | 0.41               | 0.54                 | 0.52   | FxT    | 0.893        | FxC    | 0.389            |        |                  |                |
| Predatory                     | Wheat    | 0.57                | 0.58                | 0.40                           | 0.34               | 0.34                 | 0.44   | F      | 0.303        | C      | <b>&lt;0.001</b> | T      | 0.277            | –              |
| Nematodes                     | Barley   | 0.91                | 0.79                | 0.89                           | 0.55               | 0.67                 | 0.75   | FxT    | 0.679        | FxC    | 0.697            |        |                  |                |
| Total Invertebrates           | Mean     | 73,707              | 76,091              | 68,678                         | 44,953             | 61,216               | 64,929 | F      | 0.484        | FxT    | 0.128            | T      | <b>0.005</b>     | DD > CP        |
| Total Collembola              | Mean     | 22,893              | 38,111              | 33,817                         | 22,942             | 20,345               | 27,621 | F      | 0.211        | FxT    | 0.256            | T      | <b>0.007</b>     | DD > CP        |
| Total Acari <sup>§</sup>      | Mean     | 37,691              | 34,346              | 29,962                         | 19,923             | 30,039 <sup>*</sup>  | 29,717 | F      | 0.185        | FxT    | <b>0.039</b>     | T      | <b>0.011</b>     | DD > CP        |
| Total Other <sup>‡</sup>      | Mean     | 2189                | 1765                | 2161                           | 1174               | 1899                 | 1815   | F      | 0.352        | FxT    | 0.751            | T      | 0.552            | –              |
| Simpson's diversity           | Mean     | 0.643               | 0.691               | 0.712                          | 0.710              | 0.658                | 0.683  | F      | 0.517        | FxT    | 0.449            | T      | <b>0.004</b>     | CP > DD        |
| Entomobryomorpha <sup>‡</sup> | Mean     | 18,888              | 29,616              | 25,133                         | 14,240             | 12,802               | 19,628 | F      | 0.149        | FxT    | 0.513            | T      | <b>0.005</b>     | DD > CP        |
| Poduromorpha <sup>‡</sup>     | Mean     | 916                 | 2788                | 5257                           | 3957               | 3218                 | 3025   | F      | 0.311        | FxT    | 0.057            | T      | 0.451            | –              |
| Neelipleona                   | Mean     | 19                  | 69                  | 81                             | 116                | 64                   | 68     | F      | 0.589        | FxT    | 0.479            | T      | 0.206            | –              |
| Symphypleona <sup>‡</sup>     | Mean     | 1056                | 1622                | 892                            | 654                | 681                  | 955    | F      | 0.466        | FxT    | 0.382            | T      | <b>&lt;0.001</b> | DD > CP        |
| Mesostigmata                  | Mean     | 9,062 <sup>a</sup>  | 13,275 <sup>b</sup> | 10,826 <sup>ab</sup>           | 7,136 <sup>a</sup> | 10,679 <sup>ab</sup> | 10,196 | F      | <b>0.008</b> | FxT    | 0.329            | T      | 0.702            | –              |
| Oribatida <sup>Sy</sup>       | Mean     | 979 <sup>ab</sup>   | 1,410 <sup>b</sup>  | 241 <sup>ab</sup>              | 227 <sup>ab</sup>  | 201 <sup>a</sup>     | 466    | F      | <b>0.008</b> | FxT    | 0.273            | T      | 0.171            | –              |
| Prostigmata <sup>§</sup>      | Mean     | 26,369              | 17,394              | 17,231                         | 12,297             | 16,997 <sup>*</sup>  | 17,539 | F      | 0.392        | FxT    | <b>0.038</b>     | T      | <b>0.006</b>     | DD > CP        |

<sup>Sx</sup>, <sup>S</sup>, <sup>‡</sup>, <sup>§</sup> and <sup>Sy</sup> indicate transformation for normality as  $\log_{10}(y + 11.111)$ ,  $\log_{10}(y)$ ,  $\sqrt{\log(y)}$ , Box-Cox ( $(y^\lambda - 1)/\lambda$ ) with  $\lambda = 0.1$ , Box-Cox ( $(y^\lambda - 1)/\lambda$ ) with  $\lambda = 0.3$  and  $\log_{10}(y + 130.629)$  respectively.

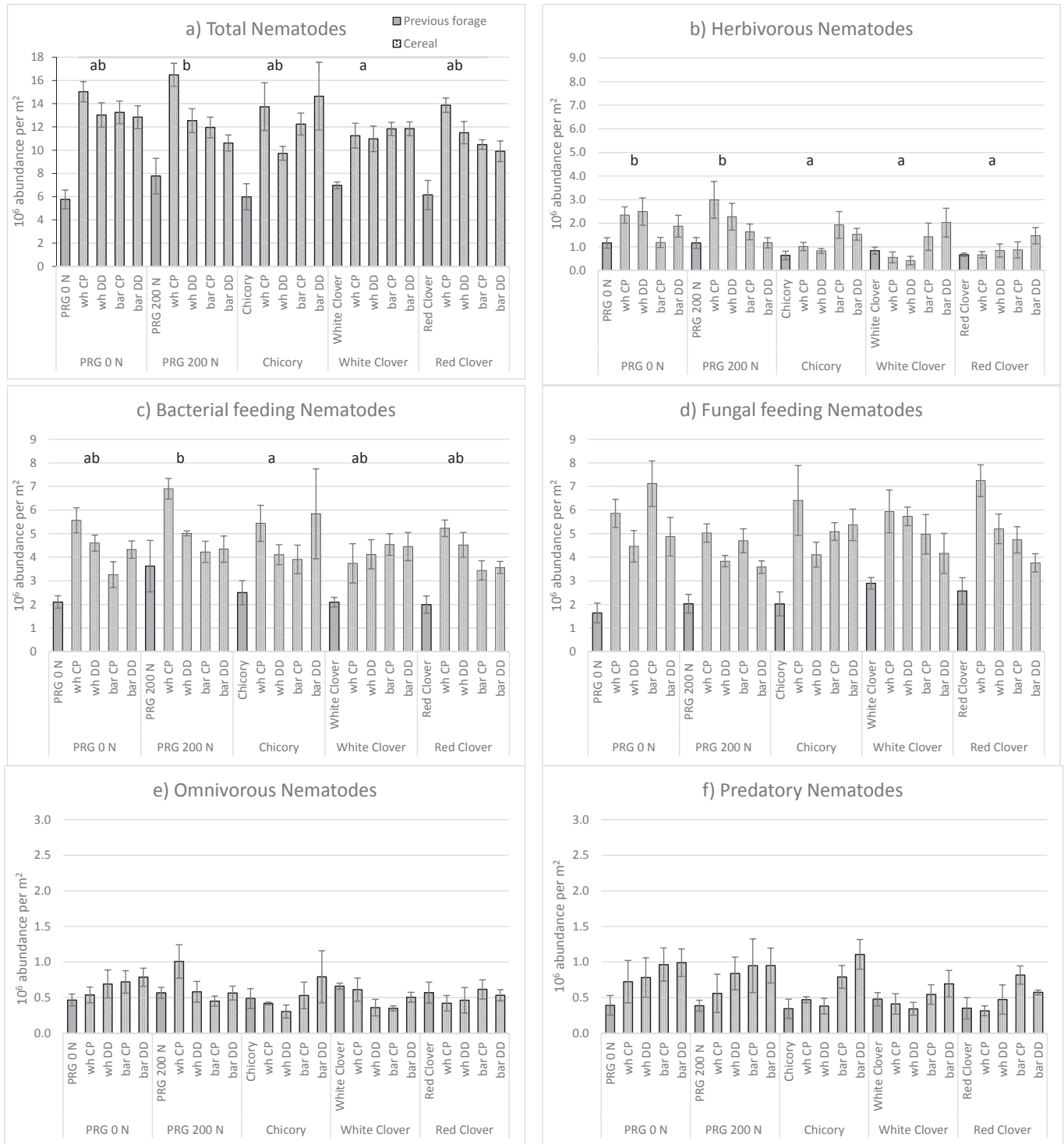
wireworms (click beetle larvae, *Elateridae* spp.) and leatherjackets (crane fly larvae, *Tipulidae* spp.), which were present in sufficient numbers to allow the effects of previous forage and tillage management to be assessed (Table 1 and Supplementary Table S.1). Overall there was a legacy effect for wireworm abundance across the previous forage treatments ( $P = 0.023$ ; Table 1 and Fig. 2(e)). After the barley crop, a significant previous forage effect on wireworm abundance was found with higher numbers in PRG low N plots than in red clover plots ( $P < 0.05$ ; Fig. 2(e)). There was no effect of tillage on wireworm abundance ( $P = 0.154$ ) but there was a trend for greater numbers of wireworms after barley compared to post wheat ( $P = 0.058$ ). Leatherjackets were more abundant post barley than post wheat ( $P = 0.001$ ). There was no evidence of a forage legacy effect, due to the very low numbers in the original previous forage treatments (Fig. 2(f)) but there was an effect of tillage method ( $P = 0.007$ ) on leatherjacket numbers with more recovered from DD plots than CP plots (Table 1 and Supplementary Table S.1). A weak negative correlation between wireworm abundance and leatherjackets was found with Spearman's rank correlation coefficient at  $P = 0.071$  for these two competing herbivore species.

### 3.2. Nematode population assessment

There was no significant difference between previous forage

treatments ( $P = 0.569$ ), or post-cereal previous forage treatments ( $P = 0.467$ ) for soil bulk density; results used to calculate nematodes per  $\text{m}^2$ . Significant differences were found for tillage method ( $P = 0.009$ ; CP  $1.12 \text{ cm}^3$  compared to DD  $1.17 \text{ cm}^3$  (results not shown)). Tillage method was one of the main effects found on nematode abundance ( $P = 0.001$ ; Table 1 and Supplementary Table S.2). However, the pattern was contrary to earthworms, with higher numbers found in the CP plots in comparison to the DD plots. Nematodes, again presenting the opposite pattern to earthworms, increased in number in the cereal rotation in comparison to the previous forage (Fig. 3(a)). There were greater numbers of earthworms in the previously ryegrass 200N treatment in comparison to WC ( $P = 0.052$ ). Whilst a forage  $\times$  crop interaction showed that the legacy effect was greatest after wheat but had diminished after barley ( $P = 0.028$ ; Table 1 and Supplementary Table S.2). Furthermore, a tillage  $\times$  crop interaction showed that, the differences between tillage treatments were most likely temporal, with the greatest differences after wheat, whilst the tillage treatments were more similar after barley ( $P = 0.028$ ; Table 1 and Supplementary Table S.2).

There was a previous forage effect post wheat on herbivorous nematodes, with significantly higher numbers found on both ryegrass treatments compared to other forage treatments ( $P = 0.005$ ; Table 1 and Fig. 3(b)) but these effects had reduced post barley as shown by a forage  $\times$  crop interaction ( $P < 0.001$ ;



**Fig. 3.** Change in abundance over time per m<sup>2</sup> (data untransformed; presented as 10<sup>6</sup> mean  $\pm$  standard error) for total nematodes (a), plant feeding nematodes (b), bacterial feeding nematodes (c), fungal feeding nematodes (d), omnivorous nematodes (e) and predatory nematodes (f) post forage post wheat ploughed (wh CP) and direct drilled (wh DD) and post barley ploughed (bar CP) and direct drilled (bar DD). Multiple comparisons based on bonferroni adjusted multiple comparisons ( $P < 0.05$ ). a, b indicate forage means differ within crop (CP and DD combined).

Table S.2). Bacterial feeding nematodes (Fig. 3(c)) showed a significant effect of previous forage but only after wheat ( $P = 0.042$ ; Table 1) with greater numbers in the previously PRG 200N compared to WC; furthermore, a tillage  $\times$  crop interaction showed that, in CP plots only, there were higher numbers post wheat compared to post barley ( $P = 0.021$ ). Fungal feeding nematodes

(Fig. 3(d)) did not show a significant relationship to previous forage (although trended towards greater numbers in previously clover forages in the wheat crop compared to barley;  $P = 0.072$  and  $P = 0.089$ ; Table S.2). Omnivorous nematodes were found in low, relatively stable numbers throughout the experimental sampling period (Fig. 3(e); Table 1). Predator nematode abundance (Fig. 3(f))



showed no effects of previous forage or tillage or interactions (Table 1; Table S.2) but cereal crop effects showed they were found in highest abundance post barley compared to post wheat ( $P < 0.001$ ).

### 3.3. Microarthropod populations

An average of  $78,000 \pm 6400$  microarthropods per  $m^2$  were extracted across previous forage treatments within the wheat cultivation – far greater abundances than those found in the previous forage treatments themselves (Crotty et al., 2015; Fig. 4(a)); around 54% were mites and 42% Collembola, whilst the remaining 4% classified as “other” invertebrates. The total microarthropods, Collembola, mites, or “other” invertebrate order abundance, did not differ in relation to previous forage treatments ( $P > 0.05$ ; Table 1 and Supplementary Table S.3) although all were significantly greater than previous forage (Fig. 4(b) and (c)). There were differences however, between the two tillage methods, with a higher abundance of total microarthropods, Collembola and mites in the DD compared to the CP ( $P = 0.005$ ,  $P = 0.007$  and  $P = 0.011$  respectively, Table 1). There was also a previous forage  $\times$  tillage interaction effect for mites overall; the abundance of mites in the previously RC treatments were three fold higher in the DD compared to the CP ( $P = 0.039$ ; Table S.3). Using Simpson's index of diversity, no previous forage treatment effect was found but there

was an effect of tillage method ( $P = 0.004$ ; Table S.3) with CP higher than DD.

Assessing the abundance of the main superfamilies for Collembola showed abundances did not differ in relation to previous forage treatment (Table 1 and Supplementary Table S.4). Tillage management did affect the abundance though; the DD cultivation had larger abundances than CP for both the Entomobryomorpha and the Symphypleona ( $P = 0.005$  and  $P = 0.001$  respectively; Table S.4). Differences between previous forage treatments were found for two of the three main mite lineages (Table 1). Mesostigmata were found in a higher abundance in the previously PRG 200N treatment in comparison to the previously PRG low N and WC treatments ( $P = 0.008$ ); whilst oribatid mites were found in a higher abundance in the PRG 200N treatment in comparison to the RC ( $P = 0.008$ ; Table 1 and Table S.4). Prostigmata mites did not show an effect of previous forage treatment on abundance; however, there was a tillage management effect, with higher numbers in the DD compared to the CP ( $P = 0.006$ ; Table S.4). There was also a previous forage  $\times$  tillage interaction effect for Prostigmata, due to abundances in the previously RC treatments being five times higher in the DD compared to the CP ( $P = 0.038$ ; Table 1). Due to the limited legacy effects found post wheat and the diminished legacy effects found for the other groups (earthworms and nematodes) post barley, microarthropod abundance post barley are not presented here.

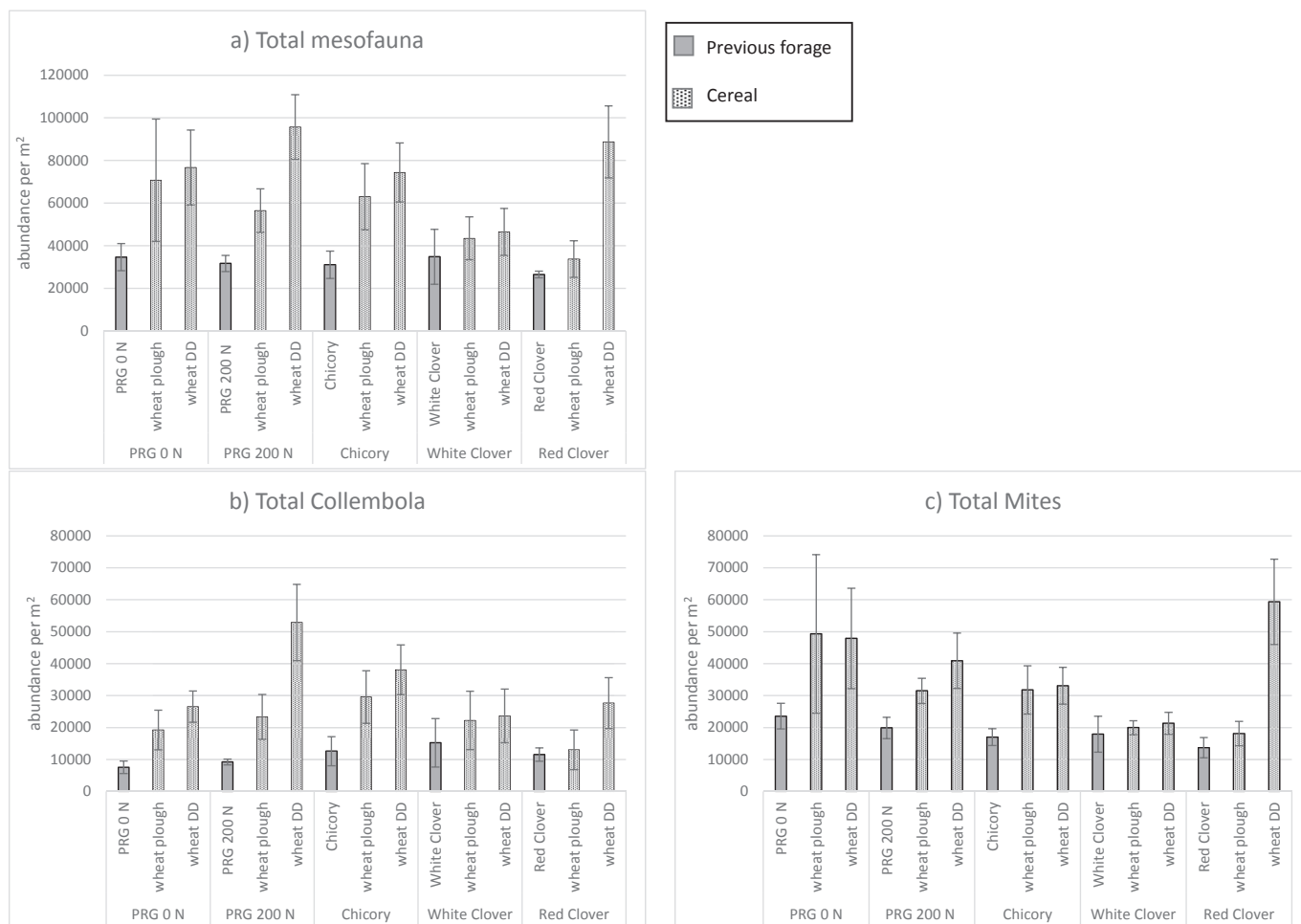


Fig. 4. Change in abundance over time per  $m^2$  (untransformed mean  $\pm$  standard error) for total mesofauna (a), total Collembola (b), and total mites (c), post forage, post wheat ploughed (wh CP) and direct drilled (wh DD) and post barley ploughed (bar CP) and direct drilled (bar DD).

#### 4. Discussion

We expect soils to perform multiple functions simultaneously, without fully understanding the impact different managements may have on the soil fauna inhabiting it. Most of the biodiversity within an agroecosystem inhabits the soil (Roger-Estrade et al., 2010). Our findings here show that there is a residual carry-over effect, or legacy, of previous forage crops which continue to create differences in the community assemblage of soil fauna after conversion to an arable crop rotation (Fig. 1). Although, Crotty et al. (2015) showed differences in community assemblage among forage treatments, the NDMS figures show a large overlap between the convex hulls, which carried over as a legacy effect into the following cereal rotation, with the convex hulls starting to diverge over time and disturbance level (CP vs DD). If there were no legacy effects, we hypothesised that these convex hulls showing abundance of functional groups would have become more similar during the cereal rotations particularly in the CP which homogenises the environment.

Our hypothesis that the original forage swards would have a legacy effect on the diversity and abundance of soil faunal populations was found. However, for the majority of fauna, the relationship was only significant in the first cereal crop within the rotation (post wheat) and had diminished by the second (post barley); possibly because the stability of the ecosystem became more important to population abundance than the system itself (Scullion et al., 2002). We hypothesised that in agreement with other studies (e.g. Chan, 2001; Kladvik, 2001; Curry et al., 2002) the DD establishment method would disturb the soil food web less than CP. Our results confirmed that tillage management did have a large effect on soil faunal populations; however, the effect was dependent on which faunal group was investigated; with faunal abundance being higher following DD for earthworms and mesofauna, whilst nematodes had higher abundances after CP. Our hypothesis that fertiliser use within the previous forages would increase the differences between treatments (ryegrass 200 N and chicory, compared to ryegrass low N, white clover and red clover), was unfounded – with the two previously ryegrass treatments being more similar to each other than the clovers, and chicory often intermediate between the two.

Previously, white clover has been found to promote earthworm abundance (Crotty et al., 2015; van Eekeren et al., 2009). Here, we have shown the legacy effects of WC persists in the following crop within a rotation (Table 1). This is conceivably due to the continued favourable soil conditions created by the WC; promoting a larger population of earthworms even after crop rotation. Reproductive rates vary among earthworm functional groups with endogeic and anecic earthworms living for more than twelve years in natural environments (even longer in culture) (Sims and Gerard, 1999), whilst epigeic earthworms have shorter lifespans on average (Mulder et al., 2007). All earthworm functional groups were affected by both previous forage and tillage management (to a lesser or greater extent) (Fig. 2), however as the significant forage × crop interactions showed that the legacy effect of previous forage was only visible one year after the forages were changed to a cereal rotation, whilst it had diminished after two years. Although these groups have different life strategies an influence of previous forage crop will continue to affect abundance. The large population decline seen across forages and tillage treatments for total earthworms and particularly epigeic earthworms was unexpected, it was hypothesised that only CP would reduce numbers to a significant extent. However, this is likely due to the reduction in plant litter available from cereal crops during the growing season in comparison to the forages (which is why a cereal-legume intercrop supports a larger population than cereal alone (Schmidt et al., 2003)).

The two most common macrofauna found in our study (other than earthworms), were both long-living herbivorous larvae (wireworms and leatherjackets) that are considered important agricultural pests (Blackshaw, 1984; Traugott et al., 2015). Legacy effects of previous forage crop were found post wheat and barley for wireworms (Table 1; Fig. 2(e)). Wireworms are known to inhabit the soil for two to four years before pupation into adults (Traugott et al., 2015), so their distribution reflects the historic oviposition site choices. However, leatherjackets have only a one year lifecycle within the soil, which may be why there is less clarity regarding the potential legacy effects. Our results also indicate a possible competitive interaction occurring between the two herbivorous species, with wireworms favouring the previously ryegrass plots and leatherjackets favouring the plots which were previously clover (Fig. 2(e) and (f)). It was not unexpected that wireworms would be more closely associated to ryegrass (Blackshaw and Hicks, 2013), however the negative Spearman's rank correlation was unexpected and requires further investigation. Both wireworms and leatherjackets are known to aggregate their oviposition sites, leading to a clustered distribution of larvae within the field (Benefer et al., 2010; Traugott et al., 2015) although this varies with species. However, due to the proximity of the previous forage crops to each other within the same field, it is improbable that the legacy effects found here are due to chance aggregation and it is more plausible that it is due to a forage species preference.

Legacy effects were found for total abundance of nematodes (post wheat and to a lesser extent post barley – Table 1 and Fig. 3) as well as for the different functional groups (bacterivorous, herbivorous and omnivorous) post wheat (Table S.2). Soil free-living and plant feeding nematodes have been found to have life spans that can vary from 7 days (e.g. *Rhabditis* spp.) to 145 days (e.g. *Ditylenchus trifurmis*) (Gems, 2000). The short lifespan of all nematodes reveals a legacy effect of previous forage treatment on the soil habitat that has had a continued effect on nematode population abundance. Total abundance of nematodes was higher in the previously ryegrass forage treatments, in comparison to the clovers post wheat (Table 1); this was noticeable for bacterial feeders and herbivores, whilst fungivorous nematodes displayed a different pattern of abundance with higher numbers in the previously clover plots (Fig. 3(b), (c) and (d)). Low levels of herbivory have been found to provide some benefits to plants by promoting root growth (Bardgett et al., 1999). However, the conceivable creation of herbivorous reservoirs within a field, as an undesirable legacy effect of previous forage with potential repercussions for future crop yields, needs further investigation.

Overall, the broad taxonomic grouping of total microarthropods did not show a forage legacy effect (Table 1; Fig. 4). Further examination of the individual lineages of mites showed some legacy effects (Table S.4), although not for the Collembola superfamilies (Table S.4). Entomobryomorpha Collembola are known to be microbivorous consuming the bacteria and fungi growing on organic matter within the soil (Crotty et al., 2014), these organisms were likely more affected by the change in environmental stability (tillage) than forage legacy. Whilst prostigmatid mites are a mixed functional group of predators, pests and fungivores, grouped due to a similarity in sucking mouth parts (Krantz and Walter, 2009), the majority of those counted within this study were the smaller species that are likely to be fungivorous. The five-fold increase in abundance of Prostigmata mites in the RC DD plots, highlights the potential interactive effects occurring between forage and tillage. This increase in abundance is likely due to a larger food source within the DD plots as these would have a more intact hyphal network, as these networks are fragmented during CP (Roger-Estrade et al., 2010). However, the significant increased abundance in the previously RC forage plots only suggests that the

stability of the environment was affected across all plots, leading to an imbalance in the soil food web, reflected by these large variances in population size. Due to the limited legacy effects found post wheat and the diminished legacy effects found for the other groups (earthworms and nematodes) post barley, microarthropod abundance post barley are not reported.

Analysing the three different scales of organisms within the soil food web, has provided us with a compartmentalised (Pokarzhevskii et al., 2003) over view of the effect agricultural management has on each group. Examination of the results of this experiment, indicate that the legacy effects reflect the function of the soil fauna monitored (Figs. 2–4). For example, the decomposer fauna (earthworms and mesofauna) are showing increased abundances in the previously leguminous forages (WC for earthworm, RC for Prostigmata mites). Whilst herbivorous fauna (nematodes and wireworms) are showing increased abundances in the previously ryegrass forage plots. The likelihood is that these changes in abundance between the invertebrates is due to food resources and adaptability to the environmental conditions. For example, high inputs of inorganic fertiliser and increased tillage promote bacterial feeding organisms (De Vries et al., 2012); this study found greater numbers of total nematodes and bacterial feeding nematodes in the PRG 200N fertiliser previous forage treatment compared to WC; greater numbers in CP compared to DD; and after a large environmental change from forage to cereal (Table 1 and Table S.2). Whilst low inputs and minimum tillage, promotes fungal feeding organisms (De Vries et al., 2012); this study found earthworms and mesofauna in the DD and previous forages which did not have inorganic N inputs (Table 1 and Tables S.1, S.3 and S.4).

A feasible explanation for these differences between previous forage treatments may be due to the original impact the forage treatments had, rather than a legacy effect *per se* in the soil. However, as this study covers soil fauna spanning a range of scales and reproductive strategies, all showing some legacy effects, it is unlikely to be due to the impact of original population sizes, and is more likely to be due to the maintenance of favourable conditions for reproduction and longevity. Dispersal rates could also have a role in the differences in population size, yet rates are known to differ between soil fauna (e.g. Oribatida 1–8 m per annum compared to earthworms moving 1 m per day (Lehmitz et al., 2012; Caro et al., 2013)), assessing populations across a range of functional groups and dispersal patterns, provides a greater insight into habitat preference and removes dispersal as a controlling factor. A related study looking at fungal communities and diversity also found there to be legacy effects within the cereal crops and that the ryegrass cultivations were the most divergent (Detheridge et al., 2016).

Soil biodiversity loss and simplification of communities impairs multiple ecosystem functions (Wagg et al., 2014), potentially reducing the soil's ability to maintain function. For example, the beneficial effects of earthworms could be used in agriculture to overcome some of the major challenges facing conventional farming (Bertrand et al., 2015) by improving soil structure, organic matter content and nutrient balances. The economic benefits of increasing earthworm numbers have also been calculated (Sandhu et al., 2008); therefore, increasing earthworm populations is of importance to soil health and agricultural profitability. In agreement with other studies, here the DD (no-tillage) method, had higher earthworm abundance than the CP treatment. Contrary to this, nematode abundance increased under CP. This is likely due to the nematodes small size, lack of permanent burrows and being able to utilise the nutrient pulse associated with the burial of crop residues making them less sensitive to ploughing than larger organisms (Kladivko, 2001). It is also possible that the greater numbers of nematodes in CP are due to the utilisation of a gap in

the ecological niches left by the reduction in earthworm numbers.

Prior to commencing this experiment, it was hypothesised that implementing a crop rotation would lead to large changes in soil fauna populations, due to the intensive management needed to grow a productive wheat crop, with for example, pastures having been shown to host larger earthworm populations than wheat fields (Bertrand et al., 2015). However, our results show that the use of different previous forage crops and different tillage management to maintain these effects are also important when considering faunal abundance overall (All figures). The interactions and inter-connectedness of soil fauna and plant-soil-fauna linkages necessitates the system being considered as a whole, to assess the impact of agriculture on the soil food web.

## 5. Conclusion

Overall our study compared the legacy effect of different forages on the abundance and diversity of soil fauna at three different scales (micro, meso and macro) during a forage-cereal rotation. The findings showed that fauna were affected at all three scales, which implies that the changes were not due to an increased initial abundance in populations prior to the cereal part of the rotation but were more likely a consequence of the residual differences the previous forages had on the soil ecosystem, maintaining different soil environments as a legacy. The tillage management also had a large effect on soil faunal abundance. However, whether DD increased abundance or not in comparison to CP was dependent on the organisms investigated. The interaction between legacy (previous forage) and tillage, was found in the wireworms (where abundance increased with time for the PRG ON DD treatments) and the Prostigmata (where there was five times higher abundance after RC in DD plots compared to the other treatments). Overall, this paper has shown there is a changing balance between disturbance, resilience and recolonization for soil fauna. Legacy effects were found across three organism scales, highlighting why the interactions and interconnectedness of the whole soil food web are needed to fully understand the impact of agriculture and to promote sustainable intensification.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2016.08.018>.

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